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The level of glycated hemoglobin (HbA_{1c}) in healthy subjects

Wartości hemoglobiny glikowanej (HbA_{1c}) u osób zdrowych

INTRODUCTION

The processes of aging and death remain one of the most fascinating, and mysterious areas of biological research. By the year 2050 one in five of the world's population will be 65 or older, the fact which presages profound medical, biological, philosophical, and political changes in the coming century [4].

One of the hypotheses trying to explain the process of aging is the idea of glycation of proteins. Ageing processes can be faster because of accumulation of toxic metabolic products of noneznymatic glycation. The synthesis of HbA_{1C} depends on the concentration of blood glucose to which the erythrocytes are exposed during their life. HbA_{1C} represents an indicator of the average concentrations of glucose during the last 3–4 months.

Glycated hemoglobin is derived from the nonenzymatic addition of glucose to the amino group of hemoglobin. HbA_{1C} is a specific glycated hemoglobin that results from the attachment of glucose to the N-terminal valine of hemoglobin β-chain [5]. They are long-living molecules which can form cross-linking between proteins and extracellular matrix [3]. There have been some reports about the relationship between age and HbA_{1C} level; however, it has been unclear. In the present study, we examined whether HbA_{1C} had any clinical significance associated with age.

MATERIAL AND METHODS

The study population consisted of 31 subject, aged 44–85, who were divided into two subgroups, one of over 60 years old. Standard phlebotomy techniques were used to obtain specimens. Samples of venous blood were collected to dry tubes without anticoagulant and they were centrifuged immediately. In the serum, concentrations of C-reactive protein (CRP), lipid profile (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides) and levels HbA_{1C} were measured using standard methods on the ACCENT 200. Determination of HbA_{1C} was based on the interaction of antigen and antibody to direct determination of the HbA_{1C} concentration in

the whole blood. The participants provided information on their age, weight and height. We calculated the body mass index (BMI) as the ratio of weight (in kilograms) to the square of height (in meters).

RESULTS

All values are expressed as mean and standard deviations (Table 1). The statistical evaluation was performed using the *t*-test for independent samples comparing the 2 proportions. The mean value of HbA_{1C} in the examined population is 4.5% without significant differences between males and females (P = 0.53). Mean value of HbA_{1C} and glucose are significant differences between the group with age under and over 60 (Fig. 1), (HbA_{1C} t = 2.68, P = 0.03) and (glucose t = 2.39, P = 0.02) (Table 1). We noticed HbA_{1C} and glucose, CRP, BMI, hemoglobin were significantly different in the whole population ($p < 0.05$). The correlation analyses of the variables were carried out using the Spearman test (Table 2). Spearman rang correlation analysis showed that in the whole group there was a significant positive correlation between HbA_{1C} and glucose ($r_s = 0.46$, P = 0.007), and there was significant positive correlation between HbA_{1C} and BMI ($r_s = 0.48$, P = 0.006). There was no correlation between age and HbA_{1C} ($r_s = 0.11$ P = 0.54) in the whole population. In the group of over 60 there was a positive correlation between HbA_{1C} and age ($r_s = 0.36$, P = 0.04) and also we noticed a positive correlation between HbA_{1C} and glucose ($r_s = 0.57$, P = 0.015), BMI ($r_s = 0.50$, P = 0.039), hemoglobin ($r_s = 0.52$, P = 0.031) (Table 2). In the group of under 60 there was no correlation with glucose, CRP, BMI and hemoglobin (Table 2). In all tests, p – value < 0.05 was considered significant. All statistical analyses were conducted using the Statistica 6.0 software.

Table 1. Baseline characteristics of healthy subjects

Parameter	All (n = 31)	< 60 (n = 14)	> 60 (n = 17)
Glucose (mg/dl)	105.25±12.27	99.85±9.70	109.70±12.63* **a**
HbA_{1C} (%)	4.51±1.30	3.97±1.20	4.97±1.22* **b**
CRP (mg/dl)	0.31±0.16	0.33±0.19	0.30±0.14
CHOL (mg/dl)	206.93±48.98	223.21±53.29	193.52±42.04
TG (mg/dl)	137.29±86.90	124.92±55.18	147.47±106.97
LDL-cholesterol (mg/dl)	110.10±39.20	127.65±44.46	95.69±28.04
HDL – cholesterol (mg/dl)	69.32±16.37	70.57±15.40	68.29±17.53
BMI (kg/m^2)	28.39±4.49	28.25±4.17	28.50±4.87
HGB (g/dl)	13.58±1.22	13.51±1.47	13.69±1.00

* P < 0.05; a – p < 0.02 in comparison with the group of under 60; b – p < 0.03 in comparison with the group of under 60

Table 2. Correlation to HbA_{1C} in healthy subjects

Correlates	Correlation coefficient the Spearman test (r_s) and P – value		
	all age (n = 31)	< 60 age (n = 14)	> 60 age (n = 17)
HbA_{1C} vs. Glucose	$r_s = 0.46$, $p = 0.007$*	$r_s = 0.13$, $p = 0.63$	$r_s = 0.57$, $p = 0.015$*
HbA_{1C} vs. CRP	$r_s = - 0.33$, $p = 0.87$	$r_s = 0.11$, $p = 0.69$	$r_s = - 0.01$, $p = 0.95$
HbA_{1C} vs. BMI	$r_s = 0.48$, $p = 0.006$*	$r_s = 0.47$, $p = 0.08$	$r_s = 0.50$, $p = 0.039$*
HbA_{1C} vs. HGB	$r_s = 0.31$, $p = 0.08$	$r_s = 0.06$, $p = 0.27$	$r_s = 0.52$, $p = 0.031$*

* $P < 0.05$

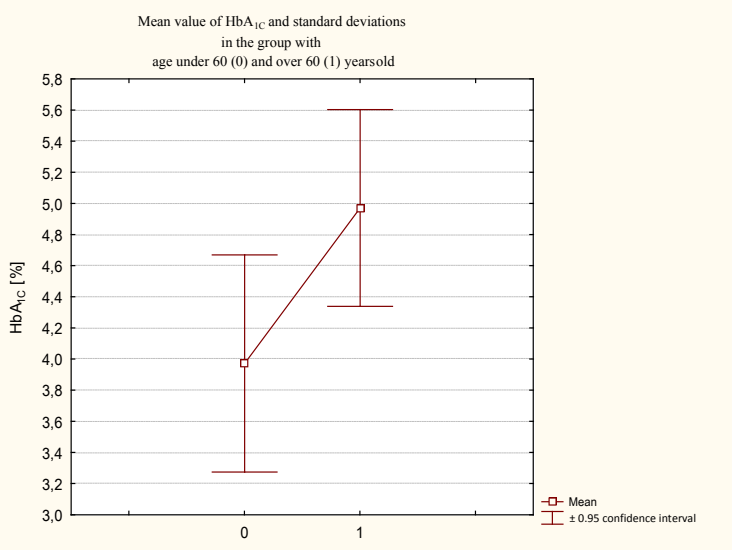

Fig. 1. Mean value of HbA_{1C} and standard deviations in the group with age under 60 (0) and over 60 (1) years old

DISCUSSION

Ageing processes can be faster because of accumulation of toxic metabolic products. These substances could be products of non enzymatic glycosylation (glycation). Glycation may therefore play an important role in ageing and has been implicated in the pathophysiology of a number of diseases, like Alzheimer disease, diabetes, kidney diseases and lung diseases [3]. In our study, HbA_{1C} was the most important risk factor for ageing processes. In agreement with the data from medical literature, we found a significant positive correlation between HbA_{1C} and glucose, CRP, BMI, hemoglobin.

As mentioned earlier, HbA_{1C} reflects blood glucose concentration over the predicting 8–12 weeks. It is in fact strictly correlated with the mean plasmatic glycemia. Glycosylation process depends on exposure, hence on the half-life of erythrocyte [2].

Obesity, especially with a central distribution of body fat, and a reduction in physical activity occur progressively with aging, and both of these factors are associated with abnormal carbohydrate metabolism [7]. The results of large cross-sectional studies have shown that both body weight and body mass index (BMI) increase throughout adult life until the age of about 50–60 years, after which they decline. A substantial minority of older people undergo a marked weight change over time [10].

Our findings are similar to those of Calisti [2] who also did not find significant differences between the mean value of HbA_{1C} and the sex in examined population, taking into account that inflammatory markers such as CRP have been related to the development of insulin resistance and 2 diabetes [9]. Higher HbA_{1C} is significantly associated with a CRP among adults. However, there was no correlation between CRP and HbA_{1C}.

Some reports have demonstrated an association of HbA_{1C} with age [5, 6, 11], whereas others have not [1, 13, 14]. In a small study of 48 subject above 50 years old, sub-divided into three age groups, Arnetz et al. observed differences in HbA_{1C} levels between the group, the oldest having the highest values. Contrary to this, Kabadi found no significant relationship between age and fasting plasma glucose (FPG), glycated hemoglobin, glycated albumin [1, 14]. Nuttal et al. [11] reported that HbA_{1C} level increased with age. Van Wersch [13] demonstrated in diabetic patients that there was a negative correlation or no relationship [12]. This might be caused by age-related nutritional, hygienic and economic factors or simply the natural course of diabetes itself. Another paper [6] revealed that the HbA_{1C} levels are positively associated with age in nondiabetic population [6].

In this study, we demonstrated a positive correlation between the group with age over 60 with HbA_{1C}. In accordance with medical literature, pathogenic mechanisms which contribute to the glucose intolerance of aging include alterations in glucose-induced insulin release and resistance to insulin-mediated glucose disposal [9]. However, old subjects have demonstrated definable alterations in glucose-induced insulin release in the aged [9]. This may be due, in part, to a decreased beta cell response to the incretion hormones. As with many hormones, insulin is secreted in a pulsatile fashion. Normal aging is also associated with subtle alterations in pulsatile insulin release, which further contribute to age-related changes in glucose metabolism [8]. Thus, it is clear that alterations in glucose-induced insulin release are an important component of the changes in carbohydrate metabolism with aging. However, the most important pathogenic mechanism underlying the glucose intolerance of aging is resistance to insulin-mediated glucose disposal [9]. There are many possible explanations for this relationship but it still has been unclear. On the other hand, Winer et al. [14] reported that they were unable to detect any direct relationship between age and HbA_{1C} measured by ion-exchange chromatography in groups of subjects carefully categorized by OGTT (Oral Glucose Tolerance Test). This discrepancy might be caused by that the evaluation of the HbA_{1C} levels still suffers from the lack of an international standard, as well as from neglecting of several cofactors like the age, gender, ethnic characteristic, interferences in the dosage, due to a condition of uraemia, hyperlipidemia, bad conservation, increase of leucocytes and presence of anomalous hemoglobins [2, 5]. For considering the values of HbA_{1C} in the elderly subjects, particular significance should be attributed to the progressive increases of glycemia with the advancing age.

CONCLUSIONS

For considering the values of HbA_{1C} in the elderly subjects, particular significance should be attributed to the progressive increases of glycemia with the advancing age. In this study, the likelihood of elevated HbA_{1C} levels increased with the levels of glucose as an effect of aging, thus glycation may play an independent role in aging (Fig. 1). These findings suggest an association between glycemic and its effect on aging.

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SUMMARY

The synthesis of HbA_{1C} depends on the concentration of blood glucose to which the erythrocytes are exposed during their life. HbA_{1C} represents an indicator of the average concentrations of glucose during the last 3–4 months. Glycated hemoglobin is derived from the nonenzymatic addition of glucose to the amino group of hemoglobin. HbA_{1c} is a specific glycated hemoglobin that results from the attachment of glucose to the N-terminal valine of hemoglobin β-chain. There have been some reports about the relationship between age and HbA_{1C} level; however, it has been unclear. In the present study, we examined whether HbA_{1C} had any clinical significance associated with age. The study population consisted of 31 subject, aged 44–85, who were divided into two groups, one of over 60 years old. Samples of venous blood were collected to dry tubes without anticoagulant and they were centrifuged immediately. In the serum, concentrations of C-reactive protein, lipid profile (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride) and levels HbA_{1C} were measured using standard methods on the ACCENT 200. Determination of HbA_{1C} was based on the interaction of antigen and antibody to direct determination of the HbA_{1C} concentration in whole blood. The mean value of HbA_{1C} in the examined population is 4.5% without significant differences between males and females. We noticed a significant positive correlation between HbA_{1C} and glucose, CRP, BMI and hemoglobin. The correlation analyses of the variables were carried out using the Spearman test. There was no correlation between age and HbA_{1C} in the whole population but in the group of over 60 the correlation was present. In this study, the likelihood of elevated HbA_{1C} levels increased with levels of glucose as an effect of aging thus glycation may play an independent role in aging. These findings suggest an association between glycemic and its effect on aging.

Key words: aging, glycemia, glycated hemoglobin

STRESZCZENIE

Glikowana hemoglobina powstaje przez przyłączanie glukozy do N-końcowego aminokwasu łańcucha β-hemoglobiny. Jest to reakcja nieenzymatyczna i odzwierciedla średnią ekspozycję hemoglobiny na glukozę w dłuższym przedziale czasowym. Glikolizacja ta może odgrywać ważną rolę w procesach starzenia i powstawania wielu chorób (Alzheimera, cukrzycy, miażdżycy, choroby nerek). Trwałe połączenia glukozy z wolną grupą aminową N-terminalnego aminokwasu białkowego lub resztą lizyny mają zdolność do tworzenia wiązań krzyżowych między białkami, co powoduje zmianę struktury macierzy międzykomórkowej. Celem podjętych badań było określenie związku między wartościami hemoglobiny glikowanej a procesem starzenia się organizmu ludzkiego. Badania przeprowadzono u 31 pacjentów w wieku 44–85 lat, których ze względu na wiek podzielono na dwie grupy: <60 ; >60 lat. Do oznaczeń parametrów biochemicznych pobrano na czczo krew żylną. W surowicy krwi oznaczono stężenie glukozy, białka C-reaktywnego (CRP), cholesterol całkowity (TC) triglicerydy (TG), HDL–cholesterol oraz LDL–cholesterol. Do bezpośredniego określenia stężenia HbA_{1C} w krwi pełnej wykorzystano reakcję antygen–przeciwciało. Powstałe zmętnienie jest proporcjonalne do ilości HbA_{1C}. W analizie statystycznej uzyskanych rezultatów badań nie

wykazano istotnych różnic w wartościach hemoglobiny glikowanej w grupie kobiet i mężczyzn. Analiza korelacji Spearmana uzyskanych wyników HbA_{1C} oraz wieku badanych osób wykazała brak istotnych zależności pomiędzy wartościami HbA_{1C} i wiekiem całej populacji, natomiast w grupie pacjentów powyżej 60 roku życia stwierdzono dodatnią zależność ze stężeniami HbA_{1C}. Stwierdzono również dodatnią korelację między wartościami BMI, CRP, glukozy, hemoglobiny a stężeniem hemoglobiny glikowanej. Zmiany towarzyszące procesowi starzenia się organizmu ludzkiego sprzyjają zaburzeniom homeostazy energetycznej i akumulacji szkodliwych produktów nieenzymatycznej glikacji białek.

Słowa kluczowe: starzenie się, glukoza, hemoglobina glikowana